IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
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Mermod	)	Atty. Dkt. **3024-119**
	)	
Appl. No. **10/595,495**	)	
	)	Examiner: n/a
	)	
Filed: 04/24/06 (National Stage)	)	Group Art Unit: n/a

For: **HIGH EFFICIENCY GENE TRANSFER AND EXPRESSION IN MAMMALIAN CELLS BY A MULTIPLE TRANSFECTION PROCEDURE OF MAR SEQUENCES**

SECOND PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Please amend the above-identified application as delineated below.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 9 of this paper.

iN the Claims:

Please amend claims 23, 44, 51and 52 and cancel claims as indicated hereinafter.

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Previously Presented) A purified and isolated DNA sequence comprising
   a) at least one bent DNA element,
   b) and at least one binding site for a DNA binding protein, wherein said DNA sequence has protein production increasing activity.

2. (Previously Presented) The purified and isolated DNA sequence of claim 1 wherein the bent DNA element contains at least 10% of dinucleotide TA, and/or at least 12% of dinucleotide AT on a stretch of 100 contiguous base pairs.

3. (Previously Presented) The purified and isolated DNA sequence of claim 2 wherein the bent DNA element contains at least 33% of dinucleotide TA, and/or at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs.

4. (Previously Presented) The purified and isolated DNA sequence of claim 1 comprising a MAR nucleotide sequence, wherein the sequence is SEQ ID Nos 1 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants.

5. – 6. (Cancelled)

7. (Previously Presented) The purified and isolated DNA sequence of claim 1, wherein said DNA binding protein is a transcription factor.

8. (Previously Presented) The purified and isolated DNA sequence of claim 7, wherein the transcription factor is a polyQpolyP domain protein.

9. (Cancelled)

10. (Previously Presented) A purified and isolated cLysMAR element and/or fragment having protein production increasing activity, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants.

11. (Cancelled)

12. (Original) A synthetic MAR sequence comprising natural MAR elements and/or fragments assembled between linker sequences.

13. (Previously Presented) The synthetic MAR sequence of claim 12, wherein the MAR sequence comprises a cLysMAR element and/or fragment, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants.

14. (Previously Presented) The synthetic MAR sequence of claim 12, wherein the linker sequences are BglII-BamHI linker.

15. (Previously Presented) A method for identifying a MAR sequence using a Bioinformatic tool comprising computing values of one or more DNA sequence features corresponding to DNA bending,
major groove depth and minor groove width potentials, and
melting temperature.

16. (Previously Presented) The method of claim 15, wherein said Bioinformatic tool contains algorithms, adapted to use of profiles or weight-matrices, to compute values for one or more of said DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials, and melting temperature.

17. (Previously Presented) The method of claim 16, wherein said profiles or weight-matrices are based on dinucleotide recognition.

18. - 22. (Canceled)

23. (Currently Amended) ~~The method~~ The method of claim 20 wherein the DNA binding protein is SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA or Vmw65 or a combination of two or more of these transcription factors.

24. (Previously Presented) The method of claim 15, wherein values for identifying DNA bending comprise between 3 to 5 °.

25. (Cancelled)

26. (Previously Presented) The method of claim 15, wherein values for the identification of the major groove depth comprise between 8.9 to 9.3 Å and values for the identification of minor groove width comprise between 5.2 to 5.8 Å.

27. (Cancelled)

28. (Previously Presented) The method of claim 16, wherein the melting temperature is between 55 to 75 °C.

29. -33. (Cancelled)

34. (Previously Presented) A method for identifying a MAR comprising
providing at least one filter detecting clusters of DNA binding sites, wherein said filter detects

said clusters using profiles or weightmatrices.

35. - 40. (Cancelled)

41. (Previously Presented) The purified and isolated DNA sequence of claim 1, comprising a sequence selected from SEQ ID Nos 24 to 27, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants.

42. (Previously Presented) A method for increasing protein production activity in a eukaryotic host cell comprising providing a purified and isolated DNA sequence comprising a first isolated matrix attachment region (MAR) nucleotide sequence, wherein the MAR nucleotide sequence is:

- a purified and isolated DNA sequence of claim 1,
- one or more sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants increasing protein production activity in a eukaryotic host cell.

43. (Previously Presented) The method of claim 42, wherein said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

44. (Currently Amended) The method of claim 43, wherein said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence comprising:

- a purified and isolated DNA sequence ~~of claim 1~~ comprising
  a.) at least one bent DNA element, b.) and at least one binding site for a DNA binding protein,
- one or more sequences of SEQ ID Nos 1 to 27,

- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence ~~of~~ comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants increasing protein production activity in a eukaryotic host cell.

45. (Previously Presented) The method of claim 44, wherein said first and at least second MAR sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest.

46. -47. (Cancelled)

48. (Original) A method for transfecting a eukaryotic host cell, said method comprising
a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one DNA sequence of interest and/or at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements,
b) subjecting within a defined time said transfected eukaryotic host cell to at least one additional transfection step with at least one purified DNA sequence comprising at least one DNA sequence of interest and/or with at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements
c) selecting said transfected eukaryotic host cell.

49. (Previously Presented) The method of claim 48, wherein said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

50. (Cancelled)

51. (Currently Amended) The method of claim 48, wherein the MAR nucleotide sequence is:
a purified and isolated DNA sequence ~~claim 1~~ comprising

a.) at least one bent DNA element, b.) and at least one binding site for a DNA binding protein,

- one or more sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants, wherein said sequence has protein production increasing activity.

52. (Currently Amended) The method of claim 48, wherein the MAR nucleotide is a purified and isolated sequence ~~according claim 1~~ comprising
a.) at least one bent DNA element, b.) and at least one binding site for a DNA binding protein, a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof and variants, wherein said sequence has protein production increasing activity.

53. - 54. (Cancelled)

55. (Previously Presented) A method for transfecting a eukaryotic host cell, said method comprising co-transfecting into said eukaryotic host cell at least one first purified and isolated DNA sequence comprising at least one DNA sequence of interest, and a second and isolated purified DNA comprising at least one MAR nucleotide selected from the group comprising:

- a purified and isolated DNA sequence of claim 1,
- one or more sequences of SEQ ID Nos 1 to 27,
- a purified and isolated cLysMAR element and/or fragment,
- a synthetic MAR sequence of comprising natural MAR elements and/or fragments assembled between linker sequences,

a sequence complementary thereof, a part thereof sharing at least 70% nucleotides in length, a molecular chimera thereof, a combination thereof or variants.

56. (Cancelled)

57. (Previously Presented) A transfected eukaryotic host cell comprising at least one purified

DNA sequence comprising at least one DNA sequence of interest and/or at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements.

58. (Previously Presented) A cell transfection mixture or kit comprising at least one purified and isolated DNA sequence according to claim 1.

59. (Previously Presented) A transgenic organism wherein at least some of its cells have stably incorporated at least one DNA sequence of claim 1.

60. - 61. (Cancelled)

62. (Previously Presented) A computer readable medium comprising computer-executable instructions for performing the method for identifying a MAR sequence claim 15.

Remarks

Claims 5 to 6, 9, 11, 18 to 22, 25, 27, 29 to 33, 35 to 40, 46 to 47, 50, 53 to 54, 56, 60 to 61 are cancelled so that claim 1 to 4, 7 to 8, 10, 12 to 17, 23 to 24, 26, 28, 34, 41 to 45, 48 to 49, 51 to 52, 55, 57 to 59 and 62 are pending in this application of which claims 1, 10, 12, 15, 34, 48 and 57 are in independent form. The above claim amendments are made to reduce the filing fee. Some minor editorial changes have also been introduced.

The amendments are not "narrowing" amendments. The scope of the claims has not been reduced; no limitations have been added and none are intended.

The disclosure has been amended to add a priority claim and a more appropriate heading.

Respectfully submitted,

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